



Fatty Acid Composition of the Freshwater Crab *Travancoriana schirnerae*

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Abstract

This study determined the fatty acid profile of claw and body meat of adult male and female *Travancoriana schirnerae*, an edible freshwater crab abundant in the wetlands of Wayanad, Kerala, India. Gas chromatography-mass spectrometry analysis of the fatty acid profile detected totally eleven fatty acids, which include saturated (SFAs) ($39.91 \pm 4.20\%$), mono (MUFAs) ($23.45 \pm 2.46\%$) and polyunsaturated fatty acids (PUFAs) ($36.63 \pm 0.97\%$). The major SFAs were arachidic (C20:0) and behenic acids (C22:0) followed by palmitic (C16:0), stearic (C18:0) and myristic acids (C14:0). The MUFAs identified were palmitoleic (C16:1) and erucic (C22:1) acids and the PUFAs include linoleic acid (C18:2 ω -6), linolenic acid (C18:3 ω -3), eicosapentaenoic acid (C20:5 ω -3) and docosahexaenoic acid (C22:6 ω -3). This study indicated that the freshwater crab *T. schirnerae* is a good source of unsaturated fatty acids, especially ω -3 (26.17%) and ω -6 (10.45%) essential fatty acids.

Keywords: Freshwater crab, body meat, claw meat, fatty acid, *Travancoriana schirnerae*

Introduction

Crabs among many other invertebrates is an important shell-fish resource (Gökoglu & Yerlikaya, 2003). Crab meat is highly nutritious owing to its content of unsaturated fatty acids (USFAs), essential amino acids and proteins (Gökoglu & Yerlikaya, 2003; Nackzk et al., 2004; Kuley et al., 2008; Sudha Devi & Smija, 2013). Apart from their commercial value for use as a human foodstuff, the biological and pharmacological role of the USFAs contained in them is of notable interest (Siscovick et al., 1995; Von

Schacky et al., 1999; Skonberg & Perkins, 2002). Crab lipids are known to contain ω (omega) -3 and ω -6 series of USFAs which reduce serum triglyceride and cholesterol level (Ayas & Ozogul, 2011). They have anti-inflammatory properties and are useful in the management of inflammatory and autoimmune diseases including rheumatoid arthritis, psoriasis, ulcerative colitis and diseases of the old age such as Alzheimer's and age related macular degeneration (Simopoulos et al., 1999; Simopoulos 2002). Franzen-Castle & Ritter-Gooder (2010) reported that ω -3 and ω -6 PUFAs are useful to the growth of retina and brain of infants and children, help in heart function and normal growth and development. Some researchers described that ω -3 fatty acids help in cancer treatments such as breast tumours (El-Sayed et al., 1984).

Lipid composition and fatty acid profiles have been extensively studied in marine crabs and other decapods (Celik et al., 2004; Cherif et al., 2008; Kaya et al., 2009; Ozogul et al., 2010; Sudhakar et al., 2011; Stanek et al., 2011). The fatty acid composition, carotenoid index and proximate analysis of the Alaskan king crab *Paralithodes camtschaticus* was carried out by Krzeczkowski et al. (2006). Rameshkumar et al. (2009) determined the fatty acid profile in edible crabs *Scylla serrata* and *Portunus pelagicus*. Kuley et al. (2008) compared the fatty acids and proximate composition of the body and claw meat of male and female blue crab *Callinectes sapidus* from different regions of the Mediterranean coast. Taufik et al. (2014) examined the fatty acid composition at the early larval stages of *P. pelagicus*. Premarathna et al. (2015) compared the nutritive value of some selected fish and crab meat and analyzed the fatty acid profile of oil extracted from *P. pelagicus*.

Freshwater crustaceans are unique in fatty acids composition and their degree of unsaturation (Bragagnolo & Rodriguez-Amaya, 2001). However,

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limited research has focused on fatty acid profiling of freshwater crab meat. The aim of this study is to investigate the fatty acid profile of claw and body meat of the freshwater crab *T. schirnerae*.

Materials and Methods

Adult intermoult males and females ($n = 10$ each) (carapace width 5.5–6 cm) were collected from the paddy fields near Mary Matha Arts and Science College, Mananthavady during August 2015. The collected specimens were brought immediately to the laboratory. Their carapace width (CW) and wet weights were recorded. Meat from the claw and body portions of male and female crabs was removed manually, collected and analysed separately for lipid and fatty acids. The total lipid from tissue was extracted with chloroform: methanol (2:1 v/v) as described by Folch et al. (1957) and estimated following the method of Frings et al. (1972).

For extraction of fatty acids profiling, the tissue was kept at 40°C in a preheated oven for two days. When the tissue was completely dried, it was ground to a fine powder. To 1 g powder, 1 ml saponification reagent (4 M sodium hydroxide in 50% aqueous methanol) was added, vortexed for 10 s and placed in a boiling water bath for 30 min and cooled. Methylation reagent (6 N HCl in aqueous methanol) 2 ml was added after vortexing for 10 s and the mixture was then placed in a water bath maintained at 80°C for 10 min. After cooling, 1.25 ml of HPLC grade hexane-methyl tertiary butyl ether mixture (MTBE (1:1 v/v) was added and centrifuged for 10 min. To the top phase, 3 ml 0.3 M sodium hydroxide was added and centrifuged for 5 min. Two-third of the upper phase was removed and used as sample for gas chromatography (Sasser, 1990).

The fatty acid profiles of claw and body meat of adult male and female crabs were analyzed by gas

chromatography-mass spectrometry (GC-MS) following the method of Nichols et al. (1995). The fatty acid-methyl esters of the samples were injected into a Chemito (GC 8610) gas chromatograph with BPX 70 capillary column coated with 5% silicone at a temperature range of 170°-310°C. Fatty acids were identified by comparison with retention times of their reference standards consisting of a mixture of SFAs and USFAs. Data obtained were analysed by Student's t-test.

Results and Discussion

The results of total lipid and profiles of fatty acids in claw and body meat of male and female crabs were presented in Tables 1-3. Claw meat possessed a considerably higher fat content than that reported for body meat (Table 1). Student's t-test established a significant difference in lipid levels between claw and body meat ($p < 0.05$).

A total of eleven fatty acids were detected which include both SFAs and USFAs (Table 2).

Saturated fatty acids ($39.91 \pm 4.20\%$) identified were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0) and behenic acid (C22:0). Arachidic and behenic acids were the dominant SFAs, representing 13.92% (12.92-15.62) and 13.28% (10.43-15.50) of the total fatty acids, respectively. The average SFA concentration was comparatively lower in females ($37.96 \pm 6.0\%$) than males ($41.86 \pm 1.32\%$); lower in the claw ($38.26 \pm 6.42\%$) than the body meat ($41.56 \pm 0.91\%$) (Table 3). However, these differences in concentrations of SFAs between the sexes and between the body parts analyzed were not significant statistically ($p > 0.05$).

The USFAs in *T. schirnerae* meat ($60.08 \pm 4.20\%$) comprised of MUFAs ($23.45 \pm 2.46\%$) and PUFAs ($36.63 \pm 0.97\%$). The mean USFA content of female crab meat ($62.03 \pm 6.00\%$) was significantly greater

Table 1. Total lipid content (mean $\pm$ SD) of claw and body meat in male and female *Travancoriana schirnerae*

Crab	Number	CW (cm)	Wet weight of body (g)	Lipid (mg 100 g ⁻¹ wet weight of tissue)	
				Claw meat	Body meat
Male	10	5.5-6.0	43.28-57.56	401.0-580.0	264.0-284.0
		5.61 $\pm$ 0.15	48.93 $\pm$ 4.66	490.5 $\pm$ 126.5	274.0 $\pm$ 14.14
Female	10	5.5-5.8	38.26-54.6	406.0-580.0	188.0-232.0
		5.59 $\pm$ 0.11	44.44 $\pm$ 4.92	493.0 $\pm$ 123.0	212.0 $\pm$ 22.27

than the male crab meat ($58.14 \pm 1.32\%$) ($t=2.27$, $p<0.05$). Though the mean USFA level of claw meat ($61.74 \pm 6.42\%$) was higher than the body meat ($58.43 \pm 0.91\%$), it was not significant statistically ($p>0.05$) (Table 3).

Erucic acid (C22:1) (ω -9) was the major MUFA (12.25 – 13.44% in male and 12.74 – 14.21% in female meat) followed by palmitoleic acid (C16:1) (ω -7) (8.30 – 8.75% in male and 11.88 – 12.25% in female meat) (Table 2). Significant differences ($p<0.05$) were indicated in terms of MUFA contents of male

and female crab meat whereas no significant difference was recorded for MUFA levels between claw and body meat ($p>0.05$).

The PUFAs present in *T. schirnerae* meat were linoleic acid (C18:2) (ω -6), linolenic (C18:3) (ω -3) acid, eicosapentaenoic acid (EPA) (C20:5 ω -3) and docosahexaenoic acid (DHA) (C22:6 ω -3) and their concentrations were found nearly equal in males ($36.77 \pm 1.85\%$) and females ($36.49 \pm 5.22\%$). Linolenic acid was the major PUFA followed by linoleic acid, representing 13.48 and 10.45% respectively of the

Table 2. Fatty acid profile (%) of claw and body meat in adult male and female *Travancoriana schirnerae*.

Fatty acids	Carbon atom	Male		Female		Average
		Claw	Body	Claw	Body	
Saturated fatty acid						
Myristic acid	C14:0	2.78	1.16	0.82	1.12	1.47
Palmitic acid	C16:0	4.20	4.36	3.48	3.42	3.86
Stearic acid	C18:0	9.96	6.89	6.07	6.55	7.36
Arachidic acid	C20:0	13.95	13.20	12.92	15.62	13.92
Behenic acid	C22:0	11.91	15.31	10.43	15.50	13.28
Monounsaturated fatty acid						
Palmitoleic acid	C16:1	8.30	8.75	11.88	12.25	10.29
Erucic acid	C22:1	13.44	12.25	14.21	12.74	13.16
Polyunsaturated fatty acid						
Linoleic acid	C18:2	9.75	9.78	11.31	10.96	10.45
Linolenic acid	C18:3	13.86	13.61	13.09	13.39	13.48
Eicosapentaenoic acid	C20:5	6.37	8.29	9.36	4.14	7.04
Docosahexaenoic acid	C22:6	5.48	6.40	6.43	4.31	5.65

The data given for individual fatty acids are percentages of total fatty acids

Table 3. Fatty acid composition of claw and body meat of adult male and female *Travancoriana schirnerae*.

	Male Crab meat	Female	Claw	Body
SFA	41.86 ± 1.32 39.91 ± 4.20	37.96 ± 6.00	38.26 ± 6.42	41.56 ± 0.91
MUFA	21.37 ± 0.52 23.45 ± 2.46	25.54 ± 0.77	23.91 ± 3.07	22.99 ± 2.82
PUFA	36.77 ± 1.85 36.63 ± 0.97	36.49 ± 5.22	37.82 ± 3.34	35.44 ± 3.73
USFA	58.14 ± 1.32 60.08 ± 4.20	62.03 ± 6.00	61.74 ± 6.42	58.43 ± 0.91

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; USFA: Unsaturated fatty acid

Each value is mean $\pm$ SD of ten individual crabs; SFA and USFA values are expressed as percentages of total fatty acids.

total fatty acids. Though slightly greater values were observed for the PUFA contents of claw ($37.82 \pm 3.34\%$) than the body meat ($35.44 \pm 3.73\%$), there were no significant differences in the PUFA levels of claw and body meat neither in male nor in female crab meat ($p > 0.05$) (Table 3).

Generally, freshwater species are rich in SFAs and low in USFAs (Edirisinghe et al., 1998) while marine species are rich in USFAs and low in SFAs (Kaya et al., 2009). Our results are in agreement with the above findings. Saturated fatty acids represented a major proportion of the fatty acid profile of *T. schirnerae* meat as reported for *Scylla tranquebarica* (Thirunavukkarasu, 2005). Meanwhile, only a minor proportion of the fatty acid profile was represented by SFAs in *Carcinus maenas* (18.1–20.7%) (Nackzk et al., 2004), *S. serrata* (23–26%) (Anas et al., 2009), *Callinectes sapidus* (23.3–24.8%) and *P. pelagicus* meat (24.7–24.9%) (Ozogul et al., 2010).

In the present study, the SFA level of male crab meat was slightly higher than the female. On the other hand, it has been reported that the SFA level in the carapace meat of male *P. pelagicus* was similar to that of female crab (Ayas & Ozogul, 2011). In *T. schirnerae*, the percentage of SFA recorded was higher in body meat than the claw meat as reported by Kuley et al. (2008) for *C. sapidus*. Arachidic and behenic acids were the dominant SFAs in *T. schirnerae* meat whereas palmitic acid was the major SFA in *Eriphia verrucosa* (Kaya et al., 2009) and *S. serrata* meat (Anas et al., 2009). The prime SFAs included were palmitic and stearic acids in *C. sapidus* (Kuley et al., 2008), *C. mediterraneus* (Cherif et al., 2008) and *Podophthalmus vigil* meat (Sudhakar et al., 2011).

The results of this study showed that *T. schirnerae* meat contained moderate amounts of MUFAs. However, the fact that females had greater quantities of these fatty acids than males indicates their nutritional value. The MUFA content of *T. schirnerae* meat is comparable to those obtained for *C. maenas* (Nackzk et al., 2004), *E. verrucosa* (Kaya et al., 2009), *P. pelagicus* (Ozogul et al., 2010) and *S. serrata* (Anas et al., 2009) and the freshwater prawn *Macrobrachium rosenbergii* (Bhavan et al., 2010). However, MUFAs represented a minor proportion of the fatty acid profile of the spider crab *Maja brachydactyla* (Marques et al., 2009) and a major proportion of the fatty acid composition (49.8%) of *Eriocheir sinensis* meat (Chen et al., 2007).

In *T. schirnerae*, erucic acid was the dominant MUFA followed by palmitoleic acid. On the other hand, oleic acid was the major MUFA in *C. mediterraneus* (Cherif et al., 2008), *E. verrucosa* (Kaya et al., 2009), *S. serrata* (Anas et al., 2009) and *E. sinensis* (Chen et al., 2007) meat. Monounsaturated fatty acids (ω -7 series) function as anti-oxidants, reduce cholesterol, prevent type II diabetes and keep the skin smooth, flexible and hydrated (Murray, 2011).

The PUFA concentration of *T. schirnerae* meat was higher than that reported for *E. verrucosa* (Kaya et al., 2009) (20.05%) and *S. tranquebarica* (Thirunavukkarasu, 2005) meat (9.81%). However, *C. maenas* (Nackzk et al., 2004) (47.1–50.5%), *P. pelagicus* (Ayas & Ozogul, 2011) (43.19–45.98%) and *S. serrata* (Anas et al., 2009) (39–43%) meat recorded higher PUFA compositions than *T. schirnerae*. Similarly, the fatty acid profiles analyzed for the spiny cheek cray fish *Orconectes limosus* (Stanek et al., 2011), *C. maenas* (Nackzk et al., 2004) and *Cancer pagurus* (Barrento et al., 2010) were dominated by PUFA. The PUFAs identified in *T. schirnerae* meat were linoleic acid (10.45%), linolenic acid (13.48%), eicosapentaenoic acid (7.04%) and docosahexaenoic acid (5.65%). On the other hand, the major PUFAs in *S. serrata* meat were eicosapentaenoic acid, docosahexaenoic acid and eicosatetraenoic acid (Anas et al., 2009). In *P. vigil*, the main PUFAs include linoleic and alpha-linoleic acids (Sudhakar et al., 2011). The highest PUFAs were eicosapentaenoic and docosahexaenoic acids in *E. verrucosa* meat (Kaya et al., 2009). Of the body parts analyzed, the total PUFA level marked slightly high in claw than the body muscle in *T. schirnerae*. Celik et al. (2004) noticed significantly higher amounts of total ω -3 and ω -6 PUFAs in claw and breast meat of *C. sapidus*. The results obtained in this study indicated that the PUFA concentrations were more or less equal in males and females, contrary to the observations of Kuley et al. (2008) in *C. sapidus*.

Polyunsaturated fatty acids have been recognized to have special pharmacological and physiological effects on human health (Siscovick et al., 1995). They help reducing the risk of coronary artery disease, maintain integrity of biological membranes and reduce serum lipids (Skonberg & Perkins, 2002). Moreover, PUFAs are used as nutraceuticals in the form of capsules or as ingredients in infant food products. The interaction and balance between ω -3 and ω -6 fatty acids are vital for the maintenance of good health. It was found that the levels of these

fatty acids were 2–7 times higher in crab meat than mussel meat (Von Schacky et al., 1999). Omega-6 fatty acids are involved in the prevention of cardiovascular diseases as they reduce cholesterol level and blood pressure (Soccol & Oetterer, 2003). Omega-9 fatty acids help reduce the risk of atherosclerosis, cardiovascular disease and stroke (Tan-Low, 1998).

In this study, the ratio of ω -3/ ω -6 fatty acids of crab meat is 2.50. Pigott & Tucker (1990) suggested that the ω -3/ ω -6 ratio is a better index for comparing the relative nutritional value of fish oils of different species. A ratio of 1:1 for ω -3/ ω -6 is considered optimal for nutritional purposes (Simopoulos, 2002). The FAO/WHO (1994) recommended that the ratio of ω -3 to ω -6 PUFA in the diet should be at least 0.1–0.2, and a higher ratio (>0.2) is more beneficial to human health. A higher ratio of ω -3 to ω -6 PUFA in the food indicates a much higher nutritional value (Chen et al., 2007; Kuley et al., 2008). In this study, the ratio of ω -3/ ω -6 PUFA was 2.76 in male and 2.27 in female crabs, indicating that both the sexes have high nutritional value. On the other hand, the ratio of ω -3 to ω -6 PUFA was 0.44 and 1.92, respectively in male and female lyophilized Chinese mitten crabs (He et al., 2012). Kaya et al. (2009) reported that the ratio of ω -3/ ω -6 was 1.84 in *E. verrucosa* meat.

Earlier study in our laboratory revealed that the meat of *T. schirnerae* is low in fat, high in protein and is a good source of amino acids (Sudha Devi & Smija, 2013). Results obtained in the present study indicate that *T. schirnerae* meat might be considered as an innovative source of essential fatty acids and therefore can be used as a source of meat for human as well as an ingredient of animal feed for aquaculture industry.

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